PATTERNS OF INTRA AND INTERSPECIFIC GENETIC DIVERGENCE IN MARINE SPONGES

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Since the first molecular systematic studies on marine sponges in the 1980's, many papers have been published about levels of allozyme divergence between conspecific and congeneric sponge populations. Those genetic studies have indicated that sponges are more divergent than other marine invertebrates, a fact that was attributed to the high levels of genetic variation and morphological conservativeness found in Porifera. However, an analysis of 55 interspecific and 87 intraspecific pairwise genetic identity (*I*) values indicates a more complex picture. This study found that the average of *I* over all interspecific comparisons (I=0.42) was not much smaller than that found among other marine invertebrates (I=0.54), and the frequency distribution of *I*, for intraspecific comparisons, appears to be bimodal. Some genera were consistently highly divergent (I<0.30; *Cinachyrella*, *Oscarella*, *Cliona*, *Spirastrella* and *Tethya*), whereas others were within the normal range of gene divergence ($0.40 < I < 0.80$; *Chondrosia*, *Suberites*, *Petrosia*, *Plakina* and *Phyllospongia*). Furthermore, in the genera *Axinella*, *Chondrilla* and *Clathrina*, both low and high levels of intrageneric genetic differentiation were found ($0.13 < I < 0.82$). This pattern may reflect a large variance in the evolutionary age of genera in sponges, with very large levels of intrageneric gene divergence for some. We conclude with two non-mutually exclusive scenarios: a) genetic identity levels are too variable among sponge species to be of any use to evaluate taxonomic rank above species, or b) the range of evolutionary divergence in some genera of sponges is so broad that they may need revision. ❐ *Porifera, gene divergence, allozymes, heterozygosity, molecular systematics, larval dispersal.*

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For marine organisms genetic markers have been extremely useful both for estimating levels of gene flow in structured populations (Burton, 1996), and for the detection of sibling species (Knowlton, 1993; Thorpe & Solé-Cava, 1994). Allozyme electrophoresis has become the method of choice for alpha (i.e. at the species level) molecular systematics of marine organisms (Thorpe & Solé-Cava, 1994; Knowlton & Weigt, 1997). The main advantage of allozyme electrophoresis for taxonomic studies is that it represents an independent set of characters for the detection of sibling species (Solé-Cava & Thorpe, 1987). Genetic markers such as allozymes are particularly powerful for alpha-taxonomy (Hillis et al., 1996) because they can be used to detect reproductive isolation in sympatry (i.e. the biological species concept of Mayr, 1981), and describe unambiguous diagnostic characters (i.e. the phylogenetic species concept of Cracraft, 1987). In addition, as they are ubiquitous, allozymes offer a yardstick to compare levels of evolutionary divergence in relation to taxonomic rank in widely different taxonomic groups. Through molecular methods, it has become easier to verify whether ichthyologists, entomologists and spongologists infer the same thing when they talk about generic taxa in their respective groups. Since 1978, over 3000 intraspecific and interspecific allozyme comparisons have been performed between marine populations (literature data based on search on the Aquatic Sciences and Fisheries Abstracts database, between 1978 and 1998). The most commonly used measure of genetic similarity is the index of gene identity (*I*; Nei, 1972), which varies from 1.0 (=complete identity) to zero. An analysis of the large database of genetic studies,

mostly for terrestrial vertebrates and *Drosophila*, demonstrated that mean levels of gene identity were, as expected, very different when conspecific populations, congeneric species or confamilial genera were compared (Thorpe, 1982; Thorpe, 1983). It was shown that less than 5% of all conspecific comparisons fell below an identity level of 0.8 (Thorpe, 1982). Consequently, the *I* value of 0.8 has been used as a threshold for deciding about specific differentiation using allozyme data to define species, especially for comparing allopatric populations, where the more straightforward use of diagnostic loci (*sensu* Ayala, 1983) is not possible, and the biological species concept (Mayr, 1981) is not practical (Aron & Solé-Cava, 1991; Claridge et al., 1997). However, that value may be still too high for making decisions about the taxonomic rank of some marine invertebrates from geographically distant populations. This is because the number of allozyme loci detectable in marine invertebrates is usually smaller than in other organisms, with a consequent increase in the variance of estimates of gene identity (Nei, 1978), and also because gene flow is expected to be limited by geographical distance, with a consequent lowering of gene identities (Palumbi, 1992). Considering that decisions about species' borders in complex groups, using genetic attributes, are best taken using what has become known as 'fuzzy logic' (Van Regenmortel, 1997), the use of a threshold value becomes very important for the comparison of allopatric sponge populations.

Allozyme electrophoresis was first employed for molecular systematics of sponge populations by Solé-Cava & Thorpe (1986) and recently for sponge population genetics (Benzie et al., 1994).

Molecular data are also very useful for inferring patterns of genetic flow linked to larval dispersal (Burton, 1996). Sponge larvae are usually short lived (e.g. Borojevic, 1970; Fry, 1971; Sarà & Vacelet, 1973), which suggests that geographical distance could determine levels of gene differentiation in sponge populations. On the other hand, the pattern of gene flow observed in many marine invertebrates is often chaotic, depending mostly on rare but long-ranging broadcasting events (Johnson & Black, 1984). It would be interesting, therefore, to verify whether gene flow among sponge populations is also chaotic or supports the 'isolation by distance' model of genetic differentiation (Wright, 1978).

It has been suggested that Porifera might display much higher levels of interspecific gene divergence than other invertebrates, possibly due to the presence, in the former, of high levels of gene variation (Solé-Cava et al., 1991a; Klautau et al., 1994; Boury-Esnault et al.,1999). If this is true, then a re-calibration of the threshold value of conspecific gene identity should be performed, in order to reduce possible type I errors (i.e. deciding that putative species are different when they are not), due to a shift in gene identities between sponge populations in relation to other organisms. This calibration would be fundamental both for the analysis of evolutionary rates in the Porifera and for the continuing study on putative cosmopolitanism in the group.

The aims of this paper are to: 1) correlate levels of intraspecific gene identity with geographical distance, in order to estimate the importance of larval dispersal to the composition of sponge populations; 2) verify whether patterns of interspecific gene similarity in sponges are indeed different from those of other marine invertebrates; and 3) re-evaluate the threshold gene identity value for making taxonomic decisions for sponges.

MATERIALS AND METHODS

Data were gathered from the literature and from unpublished studies made by our laboratory (see references listed in the table legends). Whenever necessary, values of mean heterozygosity and genetic identity (Nei, 1978) were calculated from tables of gene frequency. Geographical distances were measured as the shortest distances by sea, using a large scale map (1 cm=60km; Christie et al., 1995). The possible relationship between pairwise geographical and genetic distances for intraspecific populations was tested using a Mantel test, with 1,000 replicates (Sokal & Rohlf, 1995). Pooled data of pairwise gene identity measures of intraspecific, interspecific and intergeneric comparisons were used to construct frequency histograms, in a similar way as those built by Thorpe (1982, 1983). The significance of differences between mean identity levels in interspecific (intrageneric) and intergeneric comparisons was tested using a Mann-Whitney U test (Sokal & Rohlf, 1995).

RESULTS

From all available data, 87 intraspecific, 55 interspecific and 8 intergeneric comparisons were compiled (Tables 1-3 respectively). No significant correlation (Mantel test; $P>0.40$) was found

TABLE 1. Levels of gene identity between conspecific populations. Key: Km, distance in kilometers; NL, number of loci; *I*, unbiased mean genetic identity (Nei, 1978); H, mean Hardy-Weinberg expected heterozygosity (Nei, 1972). References: 1, Benzie et al. (1994); 2, Klautau et al. (in press); 3, Cristiano Lazoski (unpublished results); 4, Solé-Cava et al. (1992); 5, Boury-Esnault et al. (1992); 6, Bavestrello & Sarà (1992); 7, Boury-Esnault et al. (1999); 8, Sarà et al. (1992).

Species	Locality 1	Locality 2	Km	NL	*I*	h	Ref
1. *Carteriospongia flabellifera*	Willis Island (Aust)	Middle Island	8.7	6	1.00	0.19	1
	Willis Island	Magdelaine	44	9	0.90	0.26	1
	Middle Island (Aust)	Magdelaine	52	9	0.89	0.27	1
	Lihou NE (Aust)	Lihou SW	80	9	0.93	0.22	1
	Magdelaine (Aust)	Lihou SW	175	9	0.69	0.28	1
	Magdelaine	Lihou NE	200	9	0.77	0.22	1
	Willis Island	Lihou SW	210	9	0.59	0.25	1
	Middle Island	Lihou SW	210	9	0.62	0.21	1
	Willis Island	Lihou NE	245	9	0.83	0.20	1
	Middle Island	Lihou NE	245	9	0.87	0.16	1
2. *Chondrilla nucula*	Marseille (Fr)	Ligurian (It)	350	9	0.91	0.11	2
3. *Chondrilla* sp.3	Anjos (Braz)	Praia do Forno	2	9	0.89	0.27	2
	Búzios (Braz)	Anjos	44	9	0.87	0.56	2
	Búzios	Praia do Forno	44	9	0.95	0.56	2
	Itacuruca (Braz)	Picinguaba	80	9	0.95	0.24	2
	Búzios	Itacuruca	240	9	0.95	0.30	2
	Anjos	Itacuruca	240	9	0.92	0.27	2
	Praia do Forno (Braz)	Itacuruca	240	9	0.93	0.30	2
	Picinguaba (Braz)	Ilha do Mel (Braz)	280	9	0.91	0.22	2
	Anjos	Picinguaba	300	9	0.95	0.22	2
	Praia do Forno	Picinguaba	300	9	0.84	0.25	2
	Búzios	Picinguaba	310	9	0.88	0.25	2
	Itacuruca	Ilha do Mel	340	9	0.88	0.27	2
	Anjos	Ilha do Mel	560	9	0.89	0.25	2
	Praia do Forno	Ilha do Mel	560	9	0.98	0.27	2
	Búzios	Ilha do Mel	700	9	0.95	0.28	2
	Noronha (Braz)	Búzios	2400	9	0.84	0.27	2
	Noronha	Anjos	2400	9	0.88	0.31	2
	Noronha	Praia do Forno	2400	9	0.91	0.34	2
	Noronha	Itacuruca	2600	9	0.88	0.33	2
	Noronha	Picinguaba	2700	9	0.95	0.28	2
	Noronha	Ilha do Mel	3200	9	0.84	0.59	2
4. *Chondrosia reniformis*	La Ciota (Fr)	Callelongue	17	13	1.00	0.14	3
	La Ciota	Endoume	25	13	1.00	0.16	3
	Callelongue (Fr)	Endoume	8	13	0.89	0.12	3
	La vesse (Fr)	Endoume	30	13	0.95	0.11	3
	La vesse	La Ciota	30	13	0.97	0.12	3
	La vesse	Callelongue	2	13	0.99	0.08	3
5. *Chondrosia* sp.	Bermudas	Recife	6640	13	0.89	0.27	3
	Bermudas	Búzios	8300	13	0.95	0.33	3
	Bermudas	Forno	8330	13	0.95	0.30	3
	Bermudas	Angra	8600	13	0.92	0.28	3
	Recife (Braz)	Búzios	1860	13	0.94	0.27	3

TABLE 1. Continued.

5. *Chondrosia* sp. (cont.)	Recife	Forno	1890	13	0.94	0.25	3
	Recife	Angra	2160	13	0.94	0.22	3
	Búzios	Forno	30	13	0.93	0.30	3
	Búzios	Angra	300	13	0.93	0.28	3
	Forno (Braz)	Angra	270	13	0.93	0.25	3
6. *Collospongia auris*	Willis Island	Middle Island	8.7	6	1.00	0.30	1
	Willis Island	Lihou SW	210	6	0.95	0.31	1
	Middle Island	Lihou SW	210	6	0.91	0.28	1
7. *Corticium candelabrum*	La Vesse	Riou (Fr)	25	16	0.97	0.24	4
8. *Oscarella lobularis*	La Vesse	Riou	25	16	1.00	0.11	4
	La Vesse	Riou	25	12	0.98	0.12	5
9. *Petrosia clavata*	Paraggi (It)	Zoagli (It)	2	9	0.96	0.12	6
10. *Petrosia ficiformis*	Paraggi	Zoagli	2	9	0.90	0.09	6
11. *Phyllospongia alcicornis*	Willis Island	Middle Island	8.7	6	0.96	0.38	1
	Willis Island	Holmes	210	6	0.89	0.35	1
	Willis Island	Lihou SW	210	6	0.85	0.26	1
	Middle Island	Holmes	210	6	0.86	0.34	1
	Middle Island	Lihou SW	210	6	0.87	0.32	1
	Holmes (Aust)	Osprey	300	6	0.74	0.34	1
	Holmes	Lihou SW	370	6	0.77	0.27	1
	Willis Island	Osprey	430	6	0.74	0.31	1
	Middle Island	Osprey	430	6	0.76	0.38	1
	Osprey (Aust)	Lihou SW	630	6	0.49	0.24	1
12. *Phyllospongia lamellosa*	Willis Island	Middle Island	8.7	6	0.91	0.30	1
	Diamond (Aust)	Lihou SW	50	6	0.89	0.25	1
	Lihou NE (Aust)	Lihou SW	80	6	0.93	0.26	1
	Diamond	Lihou NE	120	6	0.86	0.31	1
	Willis Island	Diamond	175	6	0.96	0.35	1
	Lihou NE	Marion	175	6	0.91	0.26	1
	Lihou SW (Aust)	Marion	175	6	0.99	0.19	1
	Willis Island	Holmes	210	6	0.96	0.34	1
	Willis Island	Lihou SW	210	6	0.85	0.27	1
	Middle Island	Holmes	210	6	0.90	0.24	1
	Middle Island	Lihou SW	210	6	0.76	0.19	1
	Diamond	Marion	220	6	0.93	0.24	1
	Willis Island	Lihou NE	245	6	0.85	0.36	1
	Middle Island	Lihou NE	245	6	0.80	0.27	1
	Holmes	Diamond	300	6	0.93	0.29	1
	Holmes	Lihou SW	370	6	0.85	0.27	1
	Willis Island	Marion	380	6	0.87	0.30	1
	Middle Island	Diamond	380	6	0.91	0.24	1
	Middle Island	Marion	380	6	0.80	0.19	1
	Holmes	Lihou NE	420	6	0.85	0.31	1
	Holmes	Marion	500	6	0.85	0.24	1
13. *Spirastrella hartmani*	San Blas 1 (Pan)	San Blas 2	1	8	0.95	0.30	7
	San Blas 1	Galeta (Pan)	100	8	0.87	0.28	7
	San Blas 2	Galeta	100	8	0.95	0.29	7
14. *T. citrina*	Marsala (It)	Torbay (GB)	3600	11	0.74	0.15	8
		Average	-	-	0.89	0.26	-

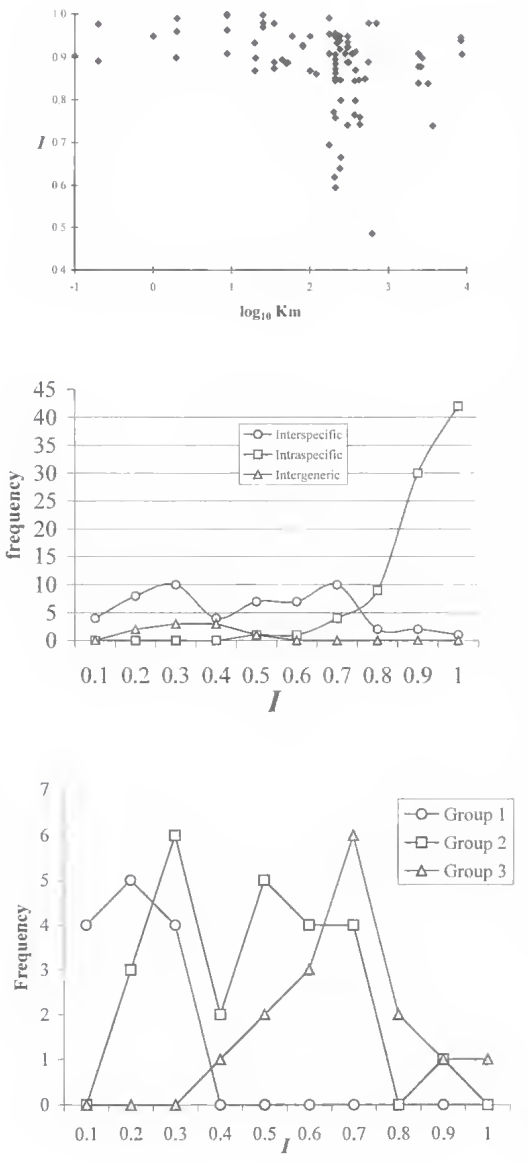

FIG. 1. A, Relationship between geographical distance (in $\log_{10}$ km) and pairwise gene identities (Mantel test; 1,000 replicates; P>0.40). B, Frequency histogram of gene identity (*I*) and taxonomic rank for species of sponges. C, Frequency histogram of gene identity (*I*) and taxonomic group. Group 1 - *Chondrosia, Suberites, Petrosia, Plakina* and *Phyllospongia*; Group 2 - *Axinella, Chondrilla* and *Clathrina*; Group 3 - *Oscarella*, *Cinachyrella*, *Tethya, Cliona* and *Spirastrella*.

between geographic distance and genetic identity (Fig. 1A).

The empirical frequency distribution of intraspecific (Table 1), interspecific (Table 2) and intergeneric (Table 3) gene identities studied on the different genera of Demospongiae and Calcarea (Fig. 1B) was similar to that found for other organisms (Thorpe & Solé-Cava, 1994). The average of *I* over all interspecific sponge comparisons was 0.42, which is similar to that found among other marine invertebrates (I=0.54). However, the distribution of interspecific pairwise gene identities in sponges was bimodal (Fig. 1C). Species of some genera were consistently highly divergent (I<0.30; 'Group 3': *Cinachyrella*, *Oscarella, Cliona*, *Spirastrella* and *Tethya*), whereas others were within the normal range of gene divergence (0.40<I<0.80; 'Group 2': *Chondrosia*, *Suberites*, *Petrosia*, *Plakina* and *Phyllospongia*). Furthermore, in the genera *Axinella, Chondrilla* and *Clathrina* ('Group 2'), species displayed both low and high levels of genetic differentiation in relation to their congeners (0.13<I<0.82). Some supposedly congeneric species had significantly lower (Mann-Whitney U test, z=2.94; P<0.004) levels of gene identity (mean I=0.16; Table 2), than species of different genera (mean I=0.30; Table 3). However, because genetic analyses have so far only focused on taxa with depauperate morphological characters or other groups presenting difficult systematic problems for Porifera, a complete pattern cannot be provided by the available data.

DISCUSSION

Two very interesting results are evident from the gene Identity analyses. 1) Generally, levels of gene identity were not correlated to geographic distance (i.e. it appears that potential for dispersal is not a key component in the structuring of sponge populations). 2) Levels of interspecific gene identities in the few sponge taxa so far examined are within the normal range found between species of other invertebrates, although some sponge genera have species that are extremely divergent from each other.

The low correlation observed between geographical distance and gene identity of intraspecific populations suggests that the length of larval life is not an essential factor in the structuring of sponge populations. Episodic recruitment events by rafting or some forms of asexual reproduction may play a more important

TABLE 2. Levels of gene identity between congeneric species. Group 1, *Chondrosia, Suberites, Petrosia, Plakina* and *Phyllospongia;* Group 2, *Axinella, Chondrilla* and *Clathrina*; Group 3, *Oscarella*, *Cinachyrella, Cliona, Spirastrella* and *Tethya*. See Table 1 for key to abbreviations. References: 1, Cristiano Lazoski (unpublished results); 2, Bavestrello & Sarà (1992); 3, Benzie et al. (1994); 4, Muricy et al. (1996); 5, Solé-Cava & Thorpe (1986); 6, Solé-Cava et al. (1991b); 7, Klautau et al. (in press); 8, Klautau et al. (1994); 9, Solé-Cava et al. (1991a); 10, Lazoski et al. (in press, this volume); 11, Barbieri et al. (1995); 12, Boury-Esnault et al. (1992); 13, Solé-Cava et al. (1992); 14, Boury-Esnault et al. (1999); 15, Sarà et al. (1992); 16, Sarà et al. (1993).

Group	Species 1	Species 2	NL	*I*	Ref
1	*Chondrosia reniformis*	*Chondrosia* sp.	12	0.48	1
1	*Petrosia ficiformis*	*Petrosia clavata*	9	0.62	2
1	*Phyllospongia lamellosa*	*Phyllospongia alcicornis*	6	0.50	3
1	*Plakina A*	*Plakina* sp.C	11	0.49	4
1	*Plakina A*	*Plakina* sp.D	11	0.73	4
1	*Plakina A*	*Plakina trilopha*	11	0.83	4
1	*Plakina C*	*Plakina* sp.D	11	0.79	4
1	*Plakina monolopha*	*Plakina* sp.C	11	0.35	4
1	*Plakina monolopha*	*Plakina* sp. D	11	0.58	4
1	*Plakina monolopha*	*Plakina trilopha*	11	0.61	4
1	*Plakina monolopha*	*Plakina* sp.A	11	0.66	4
1	*Plakina trilopha*	*Plakina* sp.C	11	0.54	4
1	*Plakina trilopha*	*Plakina* sp.D	11	0.61	4
1	*Suberites pagureorum*	*Suberites luridus*	19	0.66	5
1	*Suberites pagureorum*	*Suberites rubrus*	19	0.67	5
1	*Suberites rubrus*	*Suberites luridus*	19	0.98	5
2	*Axinella damicornis*	*Axinella verrucosa*	8	0.13	6
2	*Axinella damicornis*	*Axinella* sp.	8	0.70	6
2	*Axinella verrucosa*	*Axinella* sp.	8	0.13	6
2	*Chondrilla nucula*	*Chondrilla* sp.4 (Salvador)	9	0.23	7
2	*Chondrilla nucula*	*Chondrilla* sp.1 (Noronha)	9	0.28	7
2	*Chondrilla nucula*	*Chondrilla* sp.3 (Brazil)	9	0.33	7
2	*Chondrilla nucula*	*Chondrilla* sp.2 (Panama)	9	0.53	7
2	*Chondrilla* sp.1 (Noronha)	*Chondrilla* sp.2 (Panama)	9	0.32	7
2	*Chondrilla* sp.1 (Noronha)	*Chondrilla* sp.3 (Brazil)	9	0.48	7
2	*Chondrilla* sp.1 (Noronha)	*Chondrilla* sp.4 (Salvador)	9	0.58	7
2	*Chondrilla* sp.2 (Panama)	*Chondrilla* sp.3 (Brazil)	9	0.24	7
2	*Chondrilla* sp.2 (Panama)	*Chondrilla* sp.4 (Salvador)	9	0.25	7
2	*Chondrilla* sp.3 (Brazil)	*Chondrilla* sp.4 (Salvador)	9	0.30	7
2	*Clathrina aspina*	*Clathrina ascandroides*	9	0.57	8
2	*Clathrina aspina*	*Clathrina cylindractina*	9	0.65	8
2	*Clathrina aspina*	*Clathrina primordialis*	9	0.82	8
2	*Clathrina brasiliensis*	*Clathrina cylindractina*	9	0.43	8
2	*Clathrina brasiliensis*	*Clathrina ascandroides*	9	0.43	8
2	*Clathrina brasiliensis*	*Clathrina primordialis*	9	0.55	8
2	*Clathrina brasiliensis*	*Clathrina aspina*	9	0.69	8
2	*Clathrina cerebrum*	*Clathrina brasiliensis*	7	0.29	9
2	*Clathrina clathrus*	*Clathrina aurea*	11	0.13	9
2	*Clathrina cylindractina*	*Clathrina ascandroides*	9	0.43	8
2	*Clathrina primordialis*	*Clathrina ascandroides*	9	0.44	8
2	*Clathrina primordialis*	*Clathrina cylindractina*	9	0.65	8

TABLE 2. Continued.

3	*Cinachyrella apion*	*Cinachyrella alloclada*	19	0.27	10
3	*Cliona viridis*	*Cliona nigricans*	4	0.00	11
3	*Oscarella lobularis*	*Oscarella tuberculata*	16	0.27	12,13
3	*Spirastrella sabogae*	*S. hartmani*	8	0.12	14
3	*Tethya citrina*	*Tethya aurantium*	11	0.18	15
3	*Tethya citrina*	*Tethya norvegica*	11	0.20	15
3	*Tethya norvegica*	*Tethya aurantium*	11	0.10	15
3	*Tethya orphei*	*Tethya robusta* "B"	8	0.01	16
3	*Tethya robusta* "A"	*Tethya robusta* "B"	8	0.18	16
3	*Tethya robusta* "A"	*Tethya orphei*	8	0.27	16
3	*Tethya seychellensis*	*Tethya robusta* "B"(Red Sea)	8	0.03	16
3	*Tethya seychellensis*	*Tethya orphei*	8	0.19	16
3	*Tethya seychellensis*	*Tethya robusta* "A" (Maldives)	8	0.28	16

role in sponges, as observed in other marine invertebrates (Johnson & Black, 1984; Johnson et al., 1993; Burnett et al., 1995). This indicates that sponges follow the islands model, rather than the isolation by distance model of genetic differentiation (Wright, 1978). Levels of population structuring, measured both by pairwise gene identities (Nei, 1972) and FST inbreeding indices (Wright, 1978) are very high in sponge species (F_{ST}=0.05–0.36; Benzie et al., 1994; Klautau, unpublished results). This indicates that sponge larvae either have low capacity for dispersal, or are philopatric, as also observed for some species of ascidians (Grosberg & Quinn, 1986). In any case, the high levels of population structuring observed indicate that sponge populations are continuously diverging genetically even over small geographic scales. Possible consequences of the high level of population differentiation are the adaptation of local populations to micro-environmental conditions, and the scope for a high speciation rate in sponges (Benzie et al., 1994).

TABLE 3. Levels of gene identity between confamilial genera. See Table 1 for key to abbreviations. References: 1, Benzie et al. (1994); 2, Solé-Cava et al. (1992); 3, Guilherme Muricy & Antonio Solé-Cava (unpublished results).

Genus 1	Genus 2	NL	I	Ref
Phyllospongia	*Carterospongia*	6	0.32	1
Phyllospongia	*Collospongia*	6	0.19	1
Carterospongia	*Collospongia*	6	0.20	1
Oscarella	*Corticium*	16	0.32	2
Oscarella	*Pseudocorticium*	16	0.28	2
Corticium	*Pseudocorticium*	16	0.47	2
Plakina	*Oscarella*	11	0.22	3
Plakina	*Corticium*	11	0.30	3
Plakina	*Pseudocorticium*	11	0.40	3

In general, the frequency distribution of the values of gene identity, in relation to taxonomic rank in sponges (Fig. 1B), shows a similar pattern to that observed for other species of animals (Thorpe & Solé-Cava, 1994). The main differences observed were a slight shift to the left in the distribution of intraspecific gene identities, and the bimodal distribution of interspecific gene identities (Fig. 1B). The higher levels of intraspecific differentiation may be related to high levels of gene variation (Skibinski & Ward, 1982) as those usually observed in sponges (Solé-Cava & Thorpe, 1989; Solé-Cava & Thorpe, 1991), although no significant association between heterozygosity and gene identity was observed for the sponge data (Table 1; Spearman's Rank Correlation, P>0.10). The bimodal distribution of interspecific gene identities is more puzzling, and seems to result from different patterns of gene divergence in different sponge genera. The genera analysed can be roughly broken into three groups in relation to levels of interspecific gene identities: 1) genera whose species have similar levels of gene identity as other invertebrates (*Chondrosia, Petrosia, Phyllospongia, Plakina* and *Suberites*); 2) genera where some pairwise species comparisons give very low identity values (I<0.3), whereas others have levels of gene identity comparable to those of other organisms (0.4<I<0.8 *sensu* Thorpe, 1983; Thorpe & Solé-Cava, 1994) (*Axinella, Chondrilla* and *Clathrina*); and 3) genera where interspecies comparisons consistently give extremely low (<0.3) identity values

(*Cinachyrella, Cliona, Oscarella, Spirastrella* and *Tethya*). The three groups are significantly different from each other (Mann Whitney's U test, P<0.001 for each pairwise comparison). In relation to levels of intra and intergeneric divergence, group 1 genera showed a profile similar to that observed in other animals, that is, species of group 1 were more similar to each other than to species of other genera (Mann-Whitney's U test, P<0.0001). On the other hand, levels of gene identity between group 3 species were lower than those observed between different genera of invertebrates (Fig. 1B-C; Thorpe, 1983). More interestingly, they were also significantly lower (Mann-Whitney's U test, P<0.004) than those found between different genera of marine sponges (Table 3).

Consequently, what is the threshold gene identity value used for deciding about specific differentiation in sponge populations ? Taxonomic decisions about the comparison of morphs living in sympatry should be based on the presence of diagnostic loci (sensu Ayala, 1983; i.e. loci for which the probability of wrongly identifying one individual as belonging to one species of a pair is smaller than 1%), rather than using gene identities. The use of diagnostic loci is preferred because it is more consistent from the theoretical point of view, and decisions based on them amalgamate the power of virtually all currently accepted species concepts (Claridge et al., 1997). Diagnostic loci can, of course, also be found between allopatric populations, but in that case making taxonomic decisions about their conspecificity is not as straightforward, since allopatric populations are expected to diverge if levels of gene flow are not very large (Wright, 1978). Under the phylogenetic species concept (Cracraft, 1987), diagnostic loci indicate independent evolution, and therefore speciation. However, given the very low levels of gene flow that seem to exist between sponge populations, if we make an orthodox use of that concept we will be forced to create new species of sponges for almost every allopatric population that we analyse. A more reasonable alternative is to use, for the comparison of allopatric populations, levels of gene identity, since they take into account the overall level of divergence, on which diagnostic loci do have a heavy weight (*I* for diagnostic loci = 0), but that is less biased by episodic events of selection or drift. Given the shift to the left in the intrageneric gene identity distribution, a value of about 0.7 of gene identity can be chosen as a threshold for making decisions about interspecific differentiation between allopatric sponge populations. We chose this value because it corresponds to the point where the distribution curves of intraspecific and interspecific gene identities meet, clearly separating the two groups (Fig. 1B). If we observe the distribution of interspecific identity levels (Table 2) and consider only the comparison of sympatric populations, thus avoiding the potential circularity of using allopatric comparisons, we can see that the average of *I* is 0.52, and only 16% of the interspecific gene identity values are above 0.70. Using this threshold value, *Phyllospongia alcicornis* from Lihou and Osprey Reefs (Coral Sea, Australia), and some of the populations of *Carteriospongia flabellifera* from Lihou Reef, considered by Benzie et al. (1994) to be conspecific with those of Middle Island and Willis Island, would have to be considered as separate species (*I*=0.49-0.67).

The belief that sponge species have a higher level of genetic differentiation than other organisms (Solé-Cava et al., 1991a; Klautau et al., 1994; Boury-Esnault et al., 1999) may simply be the result of an over-representation of species of group 3 in the literature. The picture that emerges from this study using a larger set of data, indicates that levels of gene divergence among presently recognised sponge genera vary broadly, which may be the result of two different, but not mutually exclusive, phenomena. 1) Those sponge genera with genetic identities below 0.3 are so old that there has been a saturation of gene divergence leading to the accumulation of homoplasic changes (as discussed by Thorpe, 1989). 2) Some sponge genera, notably those of groups 2 and 3 above, are polyphyletic. In the first case, allozymes would be considered to be of little use above the species level in sponges, but it would remain to be explained why some sponge genera can diverge at so different rates (Solé-Cava & Thorpe, 1994). In the second instance, some sponge genera require revision, and possibly splitting up into smaller, monophyletic units. In the first case the congeneric species of group 3 should be much more different from each other than those of different genera of practically all other groups of animals (including sponges).

The high levels of gene divergence observed between conspecific sponge populations and between species in some genera of sponges should be further investigated, as they have important consequences for the taxonomic framework for the whole group. If the gene identity found

between species of *Cliona* is zero (Barbieri et al., 1995), and between species of *Spirastrella* is 0.12, what would be the identity between *Cliona* and *Spirastrella* species? Likewise, what levels of gene identity would be observed between species of *Tethya* and *Tectitethya* or *Timea* ? At this very low level of gene identity, intergeneric species may have some alleles in common by simple homoplasic convergence, due to the saturation of possible alleles detectable by the technique (Thorpe & Sole-Cava, 1994). Those convergent alleles are often found in taxonomic comparisons above the genus level, but their presence is usually detected because they conflict with a much larger number of true synapomorphies within each genus (Hillis et al., 1996). However, given the very low gene identity found between species of group 3 genera, these few convergent alleles could be misinterpreted as synapomorphies, and lead to wrong taxonomic conclusions. For example, considering the lack of synapomorphies in the molecular data within *Cliona* or *Cinachyrella*, and the possible alleles in common between species from those genera, what should be our decision about their taxonomic status? Further genetic studies, possibly linked to independent DNA analyses, are needed to determine whether allozyme data are sufficently objective to distinguish sponges at the genus level.

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LITERATURE CITED

ARON, S. & SOLÉ-CAVA, A.M. 1991. Genetic evaluation of the taxonomic status of two varieties of the cosmopolitan ascidian *Botryllus niger* (Ascidiacea: Botryllidae). Biochemical Systematics and Ecology 19: 271-276.

AVISE, J.C. 1974. Systematic value of electrophoretic data. Systematic Zoology 23: 465-481.

AYALA, F.J. 1983. Enzymes as taxonomic characters. Pp. 3-26. In Oxford, G.S. & Rollinson, D. (eds) Protein polymorphism: adaptive and taxonomic significance. (Academic Press: London).

BARBIERI, M., BAVESTRELLO, G. & SARÀ, M. 1995. Morphological and ecological differences in two electrophoretically detected species of *Cliona* (Porifera, Demospongiae). Biological Journal of the Linnean Society 54: 193-200.

BAVESTRELLO, G. & SARÀ, M. 1992. Morphological and genetic differences in ecologically distinct populations of *Petrosia* (Porifera, Demospongiae). Biological Journal of the Linnean Society 47: 61-77.

BENZIE, J.A.H., SANDUSKY, C. & WILKINSON, C.R. 1994. Genetic structure of dictyoceratid sponge populations on the western Coral Sea reefs. Marine Biology 119: 335-345.

BOROJEVIC, R. 1970. Différentiation cellulaire dans l'embryogénèse et la morphogénèse chez les spongiaires. Pp. 467-490. In Fry, W.G. (ed.) The biology of the Porifera. (Academic Press: London).

BOURY-ESNAULT, N., KLAUTAU, M., BÉZAC, C., WULFF, J. & SOLÉ-CAVA, A.M. 1999. Comparative study of putative conspecific sponge populations from both sides of the Isthmus of Panama. Journal of the Marine Biological Association of the United Kingdom 79: 39-50.

BOURY-ESNAULT, N., SOLÉ-CAVA, A.M. & THORPE, J.P. 1992. Genetic and cytological divergence between colour morphs of the Mediterranean sponge *Oscarella lobularis* Schmidt (Porifera, Demospongiae, Oscarellidae). Journal of Natural History 26: 271-284.

BURNETT, W.J., BENZIE, J.A.H., BEARDMORE, J.A. & RYLAND, J.S. 1995. Patterns of genetic subdivision in populations of a clonal cnidarian, *Zoanthus coppingeri*, from the Great Barrier Reef. Marine Biology 122: 665-673.

BURTON, R.S. 1996. Molecular tools in marine ecology. Journal of Experimental Marine Biology and Ecology 200: 85-101.

CHRISTIE, J., EWINGTON, A.M., LEWIS, H.A.G., MIDDLETON, P. & WINKLEMAN, B. 1995. The Times concise atlas of the world. (Bartholomew & Times Books: London).

CLARIDGE, M.F., DAWAH, H.A. & WILSON, M.R. 1997. Practical approaches to species concepts for living organisms. Pp. 1-15. In Claridge, M.F.H., Dawah, A. & Wilson, M.R. (eds) Species, the units of biodiversity. (Chapman & Hall: London).

CRACRAFT, J. 1987. Species concepts and the ontology of evolution. Biology and Philosophy 2: 329-346.

FRY, W.G. 1971. The biology of the larvae of *Ophlitaspongia seriata* from two north Wales populations. Pp. 155-178. In Crisp, D.J. (ed.) Fourth European marine biology symposium. (Cambridge University Press: Cambridge)

GROSBERG, R.K. & QUINN, J.F. 1986. The genetic control and consequences of kin recognition by

the larvae of a colonial marine invertebrate. Nature 322: 456-459.

HILLIS, D.M., MORITZ, C. & MABLE, B.K. 1996. Molecular Systematics. (Sinauer Associates: Massachusetts).

JOHNSON, M.S. & BLACK, R. 1984. Pattern beneath the chaos: the effect of recruitment on genetic patchiness in an intertidal limpet. Evolution 38: 1371-1383.

JOHNSON, M.S., HOLBORN, K. & BLACK, R. 1993. Fine-scale patchiness and genetic heterogeneity of recruits of the corallivorous gastropod *Drupella cornus*. Marine Biology 117: 91-96.

KLAUTAU, M., SOLÉ-CAVA, A.M. & BOROJEVIC, R. 1994. Biochemical systematics of sibling sympatric species of *Clathrina* (Porifera: Calcarea). Biochemical Systematics and Ecology 22: 367-375.

KLAUTAU, M., RUSSO, C., LAZOSKI, C., BOURY-ESNAULT, N., THORPE, J.P. & SOLÉ-CAVA A.M. 1999. Does cosmopolitanism result from overconservative systematics? A case study using the marine sponge *Chondrilla nucula*. Evolution (in press).

KNOWLTON, N. 1993. Sibling species in the sea. Annual Review of Ecology and Systematics 24: 189-216.

KNOWLTON, N. & WEIGT, L.A. 1997. Species of marine invertebrates: a comparison of the biological and the phylogenetic species concepts. Pp. 199-219. In Claridge, M.F., Dawah, H.A. & Wilson, M.R. (eds) Species, the units of biodiversity. (Chapman & Hall: London).

LAZOSKI, C., PEIXINHO, S., RUSSO, C.A. & SOLÉ-CAVA, A.M. 1999. Genetic confirmation of the specific status of two sponges of the genus *Cinachyrella* (Porifera: Spirophorida; Demospongiae) in the Southwest Atlantic. Memoirs of the Queensland Museum (this volume).

MAYR, E. 1981. Biological classification: toward a synthesis of opposing methodologies. Science 214: 510-516.

MURICY, G., SOLÉ-CAVA, A.M., THORPE, J.P. & BOURY-ESNAULT, N. 1996. Genetic evidence for extensive cryptic speciation in the subtidal sponge *Plakina trilopha* (Porifera, Demospongiae, Homoscleromorpha) from the Western Mediterranean. Marine Ecology-Progress Series 138: 181-187.

NEI, M. 1972. Genetic distance between populations. The American Naturalist 106: 283-292.

1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89: 583-590.

PALUMBI, S.R. 1992. Speciation on a small planet. Trends in Ecology and Evolution 7: 114-118.

SARÀ, M., BAVESTRELLO, G. & MENSI, P. 1992. Redescription of *Tethya norvegica* Bowerbank (Porifera Demospongiae), remarks on the genus *Tethya* in the north east Atlantic. Zoologica Scripta 21: 211-216.

SARÀ, M., CORRIERO, G. & BAVESTRELLO, G. 1993. *Tethya* (Porifera, Demospongiae) species coexisting in a Maldivian coral reef lagoon - taxonomical, genetic and ecological data. Marine Ecology 14: 341-355.

SARÀ, M., & VACELET, J. 1973. Ecologie des Démosponges. Pp. 462-576. In Brien, P., Lévi, C., Sarà, M., Tuzet, O. & Vacelet, J. (eds) Traité de Zoologie (Grassé, P. P., Sér. ed.). Vol. 3. (Masson & Cie: Paris).

SKIBINSKI, D.O.F. & WARD, R.D. 1982. Correlations between heterozygosity and evolutionary rate of proteins. Nature 298: 490-492.

SOKAL, R.R. & ROHLF, F.J. 1995. Biometry: the principles and practice of statistics in biological research. (W.H. Freeman & Co.: San Francisco).

SOLÉ-CAVA, A.M., BOURY-ESNAULT, N., VACELET, J. & THORPE, J.P. 1992. Biochemical genetic divergence and systematics in sponges of the genera *Corticium* and *Oscarella* (Demospongiae: Homoscleromorpha) in the Mediterranean Sea. Marine Biology 113: 299-304.

SOLÉ-CAVA, A.M., KLAUTAU, M., BOURY-ESNAULT, N., BOROJEVIC, R. & THORPE, J.P. 1991a. Genetic evidence for cryptic speciation in allopatric populations of two cosmopolitan species of the calcareous sponge genus *Clathrina*. Marine Biology 111: 381-386.

SOLÉ-CAVA, A.M. & THORPE, J.P. 1986. Genetic differentiation between morphotypes of the marine sponge *Suberites ficus* (Demospongiae: Hadromerida). Marine Biology 93: 247-253.

1987. The use of electrophoresis in sponge taxonomy. Pp. 243-258. In Vacelet, J. & Boury-Esnault, N. (eds) Taxonomy of Porifera from the N.E. Atlantic and Mediterranean Sea. NATO ASI Workshop Proceedings. Vol. G13 (Springer-Verlag: Berlin).

1989. Biochemical correlates of genetic variation in marine lower invertebrates. Biochemical Genetics 27: 303-312.

1991. High levels of genetic variation in natural populations of marine lower invertebrates. Biological Journal of the Linnean Society 44: 65-80.

1994. Evolutionary genetics of marine sponges. Pp. 55-63. In Soest, R.W.M. van, Kempen, T.M.G. van & Braekman, J.C. (eds) Sponges in Time and Space. (Balkema: Rotterdam).

SOLÉ-CAVA, A.M., THORPE, J.P. & MANCONI, R. 1991b. A new Mediterranean species of *Axinella* detected by biochemical genetic methods. Pp. 313-321. In Reitner, J. & Keupp, H. (eds) Fossil and Recent Sponges. (Springer: Berlin).

THORPE, J.P. 1982. The molecular clock hypothesis: biochemical evolution, genetic differentiation and

systematics. Annual Review of Ecology and Systematics 13: 139-168.

1983. Enzyme variation, genetic distance and evolutionary divergence in relation to levels of taxonomic separation. Pp. 131-152. In Oxford, G.S. & Rollinson, D. (eds) Protein polymorphism: adaptive and taxonomic significance. (Academic Press: London).

1989. Possible effects of interprotein variation in mean rate of amino acid substitution on the relationship of genetic distance with time since evolutionary divergence. Biological Journal of the Linnean Society 37: 335-344.

THORPE, J.P. & SOLÉ-CAVA, A.M. 1994. The use of allozyme electrophoresis in invertebrate systematics. Zoologica Scripta 23: 3-18.

VAN REGENMORTEL, M.H.V. 1997. Viral species. Pp. 17-24. In Claridge, M.F., Dawah, H.A. & Wilson, M.R. (eds) Species, the units of biodiversity. (Chapman & Hall: London).

WRIGHT, S. 1978. Evolution and the genetics of populations. Vol. 4. (The University of Chicago Press: London).